Plasma based Defect Engineering of Graphene for Biosensing Applications

MS042

1.0 INTRODUCTION

Diabetes is a global epidemic that plagues the lives of many. It results from the deficiency of insulin as a result of damage to the beta cells in the pancreas or due to damaged target cells, causing elevated blood glucose levels, also known as hyperglycemia. A condition rapidly rising in prevalence, diabetes is expected to affect 592 million individuals by the year 2035 [1].

Diabetes is traditionally diagnosed and monitored by the detection of glucose levels in the blood. No cure has been found for diabetes, and constant monitoring of blood glucose levels is thus required to prevent complications which results from the activation of several metabolic pathways related to inflammation and apoptosis events [2].

The possible electrode surface for glucose biosensors have been widely studied. The predominantly used platform is graphene. Graphene is a monolayer of carbon atoms arranged in a hexagonal lattice, and its physical and chemical characteristics make it ideal for applications in biosensing. Being a relatively new nanomaterial, it has spurred great interest in the field of material science due to its large surface to volume ratio and high electrical conductivity. Its peculiar characteristics can be used for biosensing, with the manufacturing of graphene-based biosensors (GBBs). The band structure of graphene can be easily modified in the presence of activating or deactivating groups which can be detected through IV characterization. This helps to amplify signals, demonstrating its promise in amperometric sensing of biomolecules [3].

Current commercially available glucose sensors typically utilise the enzyme glucose oxidase (GOx). Due to its high selectivity, GOx has an important role in the fast and accurate detection of glucose levels. The electrochemical biosensors produced with the immobilization of the enzyme can be used for a wide variety of samples. The implementation of glucose biosensors involves the anchoring of GOx onto the surface of the graphene substrate via physical adsorption. The high specific action of GOx is tapped on to catalyse the breakdown of glucose to form gluconic acid and hydrogen peroxide. The effective attachment of GOx onto the graphene substrate is essential for the larger signal to noise ratio required for proper functioning of glucose biosensors.

However, challenges remain with ensuring the efficiency of the immobilisation of GOx onto the graphene surface. The novelty of this project lies in the improvement of the attachment of GOx on graphene by using different types of plasma to engineer defects on the surface. The electrical properties (resistance) of the graphene will be monitored using an IV probe station with increasing

concentration of beta-D-glucose solutions and the quality of graphene will be ascertained using Raman spectroscopy.

1.1 Research Objective

We aimed to demonstrate that low power radiofrequency (RF) plasma exposure introduces defects on the graphene surface, which increases the surface adsorption of GOx enzyme, leading to a larger dynamic range of glucose sensing.

2.0 MATERIALS AND METHODS

2.1 Graphene synthesis by Chemical Vapor Deposition (CVD)

A 10mm x 80mm copper (Cu) strip, to be used as the growth substrate, was cleaned via sonication using isopropyl alcohol, acetone and DI water. The Cu substrate was placed on a graphite boat and placed within a 25mm diameter horizontal quartz tube which served as the CVD reaction chamber. The tube was then sealed and vacuumed to a base pressure of 3.0×10^{-3} mbar.

Hydrogen (H_2) gas with a flow rate of 2.0 standard cubic centimetres per minute (SCCM) was introduced into the tube, and subjected to a voltage of 50V via a heating element. In 20-minute intervals, the voltage was increased to 90V and 110V respectively to achieve the final temperature of around 1100°C. In this process, H_2 molecules are physically adsorbed and decompose due to the high temperature on the copper surface. The atomic hydrogen then removes any contamination on the Cu substrate, thereby allowing for a uniform growth of the graphene layer.

After 1 hour, methane gas was introduced at 10.0 SCCM. The chemisorption of methane molecules results in methane molecules being bonded to the top layer of Cu atoms, which become successively de-hydrogenised and build the honeycomb pattern of graphene. As the graphene is minorly soluble in copper, the graphene growth process is self-limiting and ceases after a monolayer is formed. After another hour, the methane supply and the heater were turned off. The reaction chamber is cooled rapidly to room temperature with the constant flow of H_2.

2.2 Graphene transfer process

Initially, the deposited graphene on the Cu substrate was transferred using the wet chemical etching method, as described in Appendix A. This method was observed to produce a non-uniform layer of graphene on the interface of the glass slide, as IV measurements failed to produce a linear graph. This was suspected to be due to the inability to remove the PMMA from the surface of graphene entirely. Subsequent transfer processes eliminated the PMMA spin-coating step, with the

graphene/Cu stack floating directly in the $FeCl_3$ solution. The remaining graphene is carefully transferred onto the glass slides and baked in the incubator. The transferred graphene samples were characterised by Raman spectroscopy.

2.3 Designing and implementing graphene-based glucose biosensors

The designing and implementation of the required device structure involved 4 major steps. First, two silver ink droplets were placed on the graphene to act as metal contacts. Second, Cu wires were fixed onto the graphene on the metal contacts. Third, the metal contacts were insulated with water-insoluble Super Glue to minimize noise resulting from current through the glucose sample. Lastly, GOx was immobilized on the graphene interface by placing 1 droplet of 1 mg/dl GOx solution onto the surface for 10 minutes, before it was soaked in DI water for 5 minutes.

2.4 Defect engineering via RF plasma

For this project, 5 different types of GBBs were tested, each with different durations of exposure to RF plasma – 0 min, 5 min, 10 min, 15 min and 20 min.

Each GBB was placed within a 40mm diameter horizontal quartz tube. Next, the quartz tube was sealed and evacuated to a base pressure of around 3.0×10^{-2} mbar. H_2 gas was introduced at a flow rate of 26.5 SCCM. After pressure reached 0.7 mbar, the capacitively coupled remote plasma was switched on at 50W. The experimental setup is shown in Appendix B. After the set period of time, the H_2 supply and RF power supply were turned off. The GBBs were then characterized with Raman spectroscopy to ascertain the presence of defects.

2.5 IV measurements

The resistance of each GBB was measured using an IV probe station. Before plasma treatment, their IV properties were ascertained using the IV probe for each increasing concentration of glucose from 10 mg/dl to 80 mg/dl. After each IV measurement, the graphene surface was washed by placing 1 droplet of DI water onto the surface for 2 minutes. IV measurements were then taken again. Different concentrations of glucose were tested with the GBBs and this process was repeated after plasma treatment.

3.0 RESULTS

3.1 Raman spectroscopy

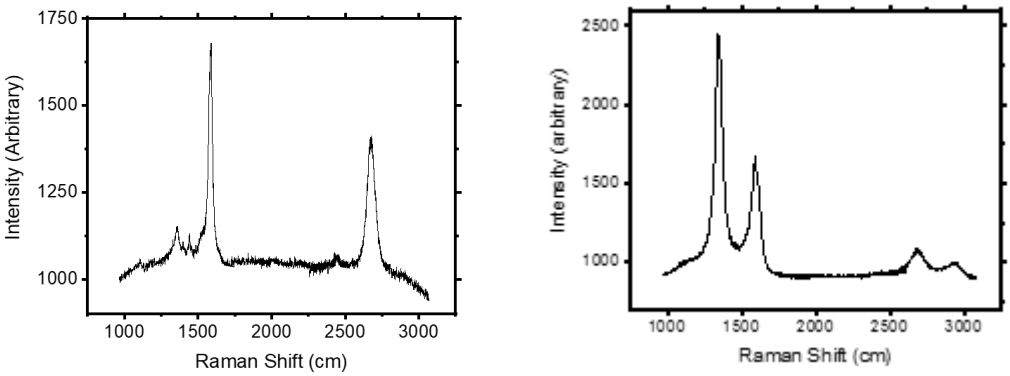

Figure 1: Raman spectra for graphene: (a) after CVD (b) after 15 min of plasma treatment

From Figure 1(a), when the graphene sample obtained by CVD was characterised via Raman spectroscopy, the characteristic "G" peak was measured to be 1587.94cm. The "G" peak's position is highly sensitive to the number of layers of graphene and is an extremely accurate way to determine the atomic thickness of the graphene. Using formula (1), it can be calculated that the graphene is a monolayer.

$$\text{Raman shift of "G" peak} = 1581.6 + 11/(1 + n^{1.6}) \quad (1)$$

The "D" peak is negligible, which shows that the graphene was of few defects before being subjected to plasma treatment. The "2D" peak is the result of a two phonon lattice vibrational process. However, unlike the D peak, it does not require defects to be activated. It can also be used to determine the thickness of the graphene layer. The "2D" peak in Figure 1(a) is sharp and symmetrical, which provides additional support that the graphene before plasma treatment is a monolayer.

From Figure 1(b), after the graphene sample had undergone 15 minutes of RF plasma treatment, there was a larger "D" peak, indicative of the presence of defects on the graphene surface. The "D" peak is known as the defect band and it represents a ring breathing mode from sp2 carbon rings within graphene, though to be active, the ring must be adjacent to a graphene edge or a defect. The peak is usually very weak in graphite and high-quality graphene. The significant "D" peak shows there were multiple defects in the material as the intensity of the "D" peak is directly proportional to the number of defects in the sample.

The "2D" peak remained symmetrical after plasma treatment, and did not split into several overlapping modes, which suggests that the graphene had remained a monolayer after RF plasma treatment. The loss of the sharp peak and the formation of a lower "2D" peak is the result of an increase in the number of defects on the graphene. The addition of the "D' + D" peak is also indicative of the presence of defects on the graphene. The "G" peak remained at 1587.94cm, indicating that the graphene remained a monolayer.

3.2 Capability of graphene for glucose sensing

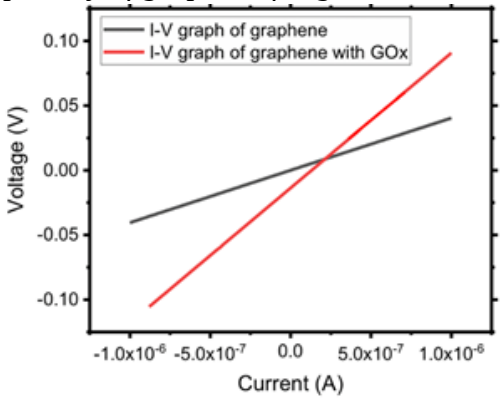

Figure 2: IV curve for GBB with and without GOx

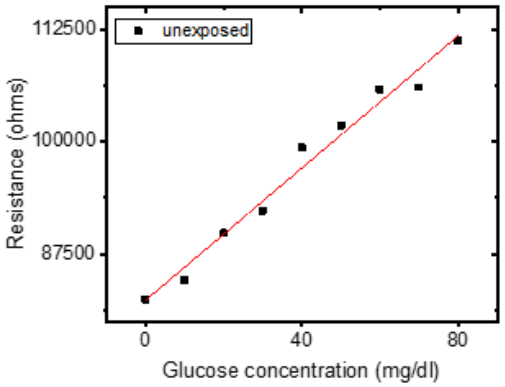

Figure 3: Change in the resistance of GBB with increasing glucose concentration

From Figure 2, the current-measured voltage (IV) graph shows that the resistance of the graphene sample before the immobilization was 40449Ω. After GOx was immobilised onto the graphene, the resistance was observed to increase to 104380Ω.

From Figure 3, the IV measurements showed that the resistance of GBBs increased linearly with increasing concentrations of glucose.

For GOx to function as a catalyst, the presence of a redox cofactor is needed — in this case flavin adenine dinucleotide (FAD) is used, which is located within the GOx molecule. [6] For this project, the mechanism justifying the linear increase in resistance is as follows:

FAD present in GOx acts as the initial electron acceptor and is reduced to $FADH_2$ according to equation (3). [7] $FADH_2$ then reacts with oxygen, forming hydrogen peroxide, in consonance with equation (2). The cofactor (FAD) is regenerated in the process, and will continue to catalyse the oxidation of other glucose molecules.

The equations are as shown below:

$$GOx\ (FADH_2) + O_2 \rightarrow GOx\ (FAD) + H_2O_2 \qquad (2)$$

$$Glucose + GOx\ (FAD) \rightarrow Gluconolactone + GOx\ (FADH_2) \qquad (3)$$

Hydrogen peroxide is a Lewis acid, with a pH of 4.5, which is due to partial dissociation of hydrogen peroxide as shown in reaction (4). The π-orbitals of graphene overlap with the π-orbitals of the GOx. The formation of the electrophilic hydroperoxyl (HO_2^-) and hydrogen (H^+) ions causes the electron cloud of graphene to skew towards the ions. The lower electron density at the surface of the graphene results in a lower number of mobile charge carriers to conduct electricity. Thus, the current is lower, leading to a higher resistance.

$$H_2O_2 \rightleftharpoons H^+ + HO_2^- \qquad (4)$$

As the reaction continues to progress, the concentration of hydroperoxyl ions within the glucose solution increases, resulting in a higher resistance.

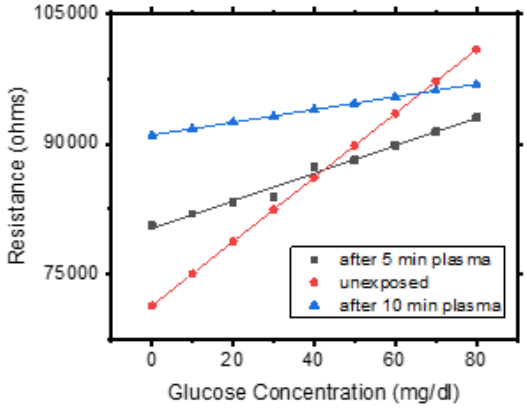

Figure 4: Resistance of GBBs after: 0, 5, 10min

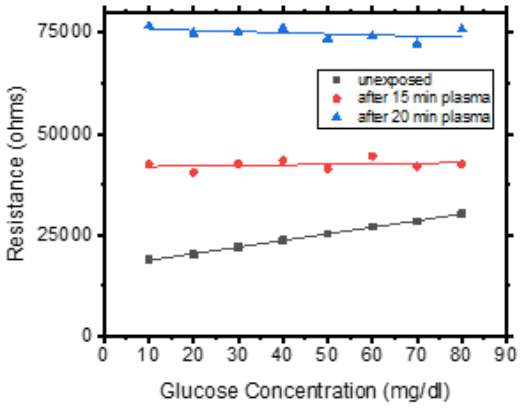

Figure 5: 15min and 20min of exposure to RF plasma

Comparing the graphs of the resistance of the GBB unexposed to plasma and that of the GBB exposed to 5 minutes of plasma in Figure 4, the initial resistance of GBB increased after the RF plasma treatment.

This can be attributed to the increase in physical adsorption of GOx onto the GBB, which resulted from the plasma-engineered defects on the graphene surface. The defects increased the surface area for the adsorption of GOx. Each GOx molecule obstructs the movement of electrons, decreasing the electron mobility of the graphene. This results in an increase in resistance. This increase continues to be observed in GBBs exposed to 10, 15 and 20 minutes, suggesting that exposure to RF plasma is effective in aiding greater immobilization of GOx on the graphene surface, increasing the area of sensitivity of the GBBs.

A comparison of the slopes of the graphs also shows that a greater duration of RF plasma treatment results in a gentler gradient. This signifies that a smaller increase in resistance for every 10 mg/dl increase in glucose concentration is observed.

However, from Figure 5, the GBBs exposed to 15 minutes and 20 minutes of RF plasma were observed to have no increment with the increased glucose concentration. This suggests that the ions in RF plasma had resulted in defects to a degree that GOx molecules were no longer able to effectively bind to the surface of graphene.

It thus follows that GBBs exposed to 5 minutes and 10 minutes of RF plasma treatment were the best candidates as effective biosensors. Although 10 minutes of plasma treatment were able to increase the immobilisation of GOx to a greater degree and is thus able to achieve a greater range of sensitivity, the lower gradient of the glucose concentration-resistance graph meant that there is a greater reliance on the specificity of GOx. As such, the GBB exposed to 5 minutes of plasma can be concluded to be the optimal GBB as it creates a balance between the range of sensitivity as well as room for error of the device.

4.0 FUTURE STUDIES

In addition to taking IV measurements, the immobilisation of GOx could be ascertained more accurately with SEM characterisation techniques as a secondary check. The exact mechanisms of how the resistance of GBBs increases with increasing glucose concentration is also not known, and future studies could test the hypotheses for the mechanisms presented in this paper. Other types of plasma and effects of plasma on other nanomaterials suitable as electrodes for GBBs could also be studied further.

5.0 CONCLUSION

GBBs have shown to demonstrate biosensing capabilities. Exposure to RF plasma is able to introduce defects on the graphene surface, as shown by Raman characterisation, which increases the surface adsorption of the GOx enzyme. This increased binding of GOx then leads to a larger dynamic range of glucose sensing. Through the measurement of the GBBs' electrical properties, the most optimal duration of RF plasma treatment was found to be 5 minutes, as it was able to demonstrate balance between a wider detection range and high sensitivity, with its detectable range far surpassing current glucose biosensors. However, future work is necessary to confirm the mechanisms present in causing the resistance of GBBs to increase with the increase in glucose concentrations.

REFERENCES

[1] Ritesh K., Lakshmana P. N., Sadishkumar K., Jayaprakash S., and Muthupillai V. (2015). Evidence for current diagnostic criteria of diabetes mellitus *World J Diabetes. 7(17): 396–405.*

[2] Scognamiglio, V. (2013). Nanotechnology in glucose monitoring: Advances and challenges in the last 10 years. *Biosens. Bioelectron.* 47, 12–25

[3] Janire Peña-Bahamonde, Hang N. Nguyen, Sofia K. Fanourakis & Debora F. Rodrigues (2018) Recent advances in graphene-based biosensor technology with applications in life sciences *Journal of Nanobiotechnology*volume 16, Article number: 75

[4] C. Shan, H. Yang, J. Song, D. Han, A. Ivaska, and Li N. (2009) Direct Electrochemistry of GOx and Biosensing for Glucose Based on Graphene *Anal. Chem. , 81,* 2378–2382

[5] A. A. Sehat , A. A. Khodadadi, F. Shemirani, Y. Mortazavi (2014) Fast Immobilization of GOx on Graphene Oxide for Highly Sensitive Glucose Biosensor Fabrication. *Int. J. Electrochem.* Sci., 10 (20145) 272 – 286

[6] Fritea, L., Tertis, M., Sandulescu, R., & Cristea, C. (2018). Enzymes–Graphene Platforms for Electrochemical Biosensors Design With Biomedical Applications. *Methods in Enzymology*. doi:10.1016/bs.mie.2018.05.010

[7] Weibel MK, Bright HJ. The GOx mechanism. Interpretation of the pH dependence. *J Biol Chem*. 1971 May 10; 246(9):2734-44.

APPENDICES

Appendix A

Polymethyl methacrylate (PMMA) was spin-coated on top of the graphene/copper and baked at 100°C for 2 minutes. The Cu layer was then removed by floating the PMMA/graphene/Cu stack in $FeCl_3$ solution for 5 hours. The remaining PMMA/graphene stack was rinsed in DI water and transferred onto a glass slide cleaned via sonication, before it was kept in an incubator for around 8 hours to remove moisture. An acetone bath was then used to remove the PMMA layer.

Appendix B

The experimental setup of plasma defect engineering is shown below.